

Prevalence and distribution of chytridiomycosis throughout Asia

By Andrea Swei, Jodi J. L. Rowley, Dennis Rödder, Mae L. L. Diesmos, Arvin C. Diesmos, Cheryl J. Briggs, Rafe Brown, Trung Tien Cao, Tina L. Cheng, Rebecca A. Chong, Ben Han, Jean-Marc Hero, Huy Duc Hoang, Mirza D. Kusri, Duong Thi Thuy Le, Jimmy A. McGuire, Madhava Meegaskumbura, Mi-Sook Min, Daniel G. Mulcahy, Thy Neang, Somphouthone Phimmachak, Ding-Qi Rao, Natalie M. Reeder, Sean D. Schoville, Niane Sivongxay, Narin Srei, Matthias Stöck, Bryan L. Stuart, Lilia S. Torres, Dao Thi Anh Tran, Tate S. Tunstall, David Vieites, & Vance T. Vredenburg

The fungal disease, chytridiomycosis, caused by the chytrid fungus *Batrachochytrium dendrobatidis* (Bd) is the greatest known disease threat to vertebrate biodiversity (Skerratt et al 2007; Wake and Vredenburg 2008). Most of the focus and research on the emergence and impact of this disease has been in Australia, USA, Europe, and Central and South America, yet very little is known about the distribution or prevalence of Bd in Asia, a region that comprises a significant portion of global amphibian biodiversity and also contains high rates of endemism. We assembled a large international team of researchers to address the Bd knowledge gap in Asia and recently published our results in PLoS ONE (Swei et al. 2011).

Targeting the keratinized epidermal cells of post-metamorphic amphibians and the mouthparts of tadpoles, Bd has already been implicated in the decline of hundreds of amphibian species and is believed to be responsible for the extinction of several species (Skerratt et al 2007; Fisher et al 2010). The Bd pathogen is characterized by its ability to invade a region and quickly spread in a linear wave-like pattern through vast geographic areas while driving whole metapopulations and species to extinction (Vredenburg et al. 2010, Lips et al. 2006).

Though Bd research in Asia has been relatively rare, the few studies that have examined Bd in Asia have either failed to find it or found low prevalence. Interestingly, there are also currently no reports of major population declines due to Bd in Asia. This pattern deviates from reports in Central America, California, Europe and Australia. Furthermore, there is growing evidence that Bd intensity on an animal must reach a minimum threshold in order to induce mortality in amphibians; this has been termed the Vredenburg 10,000 rule (Kinney et al. 2011). The few studies that have detected Bd in Asia report very low pathogen intensity (but see Savage et al. 2011).

In our recent publication (Swei et al. 2011) we proposed three hypotheses to explain why Bd is not currently linked to significant amphibian declines in Asia: 1) Bd has not fully emerged in Asia, 2) Bd is endemic to Asia and shares an evolutionary history with native host species who are resistant, and 3) Bd emergence is inhibited due to abiotic or biotic conditions that are unique to Asia. We set out to evaluate these potential hypotheses by conducting the most extensive survey of Bd in Asia to date. Over the course of nearly a decade (2001-2009) our colleagues collected 3363 samples from frogs and amphibians from 15 countries in Asia, including Papua New Guinea. Most of these samples were tested using a quantitative PCR method that can determine the infection intensity on the animal measured in zoospores counts, but many samples were also tested using histology.



Rana similis is possibly declining at Mt. Palay-palay in the Philippines and has tested positive for Bd. Photo: Rafe Brown.

We found surprisingly low infection prevalence across the samples. Only 2.35% of all the animals sampled were positive for infection with Bd. Countries where Bd was found included Kyrgyzstan, Laos, Malaysia, the Philippines, South Korea, Sri Lanka, Vietnam, and Indonesia. On average the infection intensities were less than 300 zoospores per sample, far less than the documented mortality threshold of 10,000 zoospores.

In addition, we also compared the Bd distribution results to a Maxent species distribution model that was published by Rodder et al. (2010). The Rodder et al. model was based on existing prevalence data that did not include any presence data from Asia (because it did not exist prior to their study). We found that Bd tended to occur in the areas deemed suitable by the climate model but that Bd was not detected in many other areas of Asia that the model also predicted were suitable for Bd.

These findings suggest that Bd has not (yet) caused epidemic-level declines in Asia. If the disease is invading Asia, there appear to be multiple points of introduction because the distribution of the pathogen does not follow a clear geographic pattern that would suggest a linear, wave-like spread of the disease across an entire continent. Thus if the disease is emerging in Asia, it does not appear to be spreading at the same rate as it has been documented in other regions of Bd introduction and spread. The most complete temporal record of Bd invading a naive population is in the Sierra Nevada Mountains of California. There, researchers documented the rapid spread of the disease through populations

of the mountain yellow-legged frog over the course of several seasons, with metapopulations being driven to extinction within months (Vredenburg et al. 2010). This does not appear to be the pattern in Asia where several years of sampling have not revealed population-level declines.

The second hypothesis that Bd is endemic to Asia is based on genetic evidence of an ancestral Bd haplotype in Japan (Goka et al. 2009). If Bd is native to Asia, then the current biodiversity that was sampled is a post-disease landscape with extant amphibians representing Bd-resistant lineages. However, if this were the case, disease prevalence should be much higher. The prevalence that was found throughout Asia (2.35%) could not be sustained without continued reintroduction of the pathogen from a reservoir and indicates that the disease is probably not self-sustaining. In other regions where populations are co-existing with Bd in a post-epidemic situation, prevalence is much higher (Briggs et al. 2010). Further investigations of genetic strains of Bd in Asia and elsewhere is required to establish endemism. However, even if Bd is endemic to Asia, this recent study concluded that epidemiologically, it does not explain the lack of declines there.

The last hypothesis is quite broad and encompasses a large number of factors that could explain the difference in Bd behavior in Asia. One factor that could be important is the presence of symbiotic skin microbes that have been shown to have a protective, anti-Bd effect in other amphibians (Harris et al. 2009). Currently, there have not been any studies on the microbiota of Asian amphibians and whether they have similar protective effects on hosts. The authors suggest this is an area that deserves more research.

Our study showed that Bd is broadly distributed but at very low prevalence throughout Asia, however, sites were only visited once and with no data on disease dynamics it is difficult to predict if Bd is an immediate threat to amphibian populations in Asia. To date, no epidemics have been reported but that may change as global conditions change or as the disease spreads to more susceptible populations. Because we found high prevalence and relatively high infection intensities at one site in the Philippines, we caution that this site may represent a potentially emerging site.

In summary, we conclude that Bd has apparently not yet emerged in Asia. This may be because it has not been introduced in sufficient intensity or that the epidemiology of Bd is

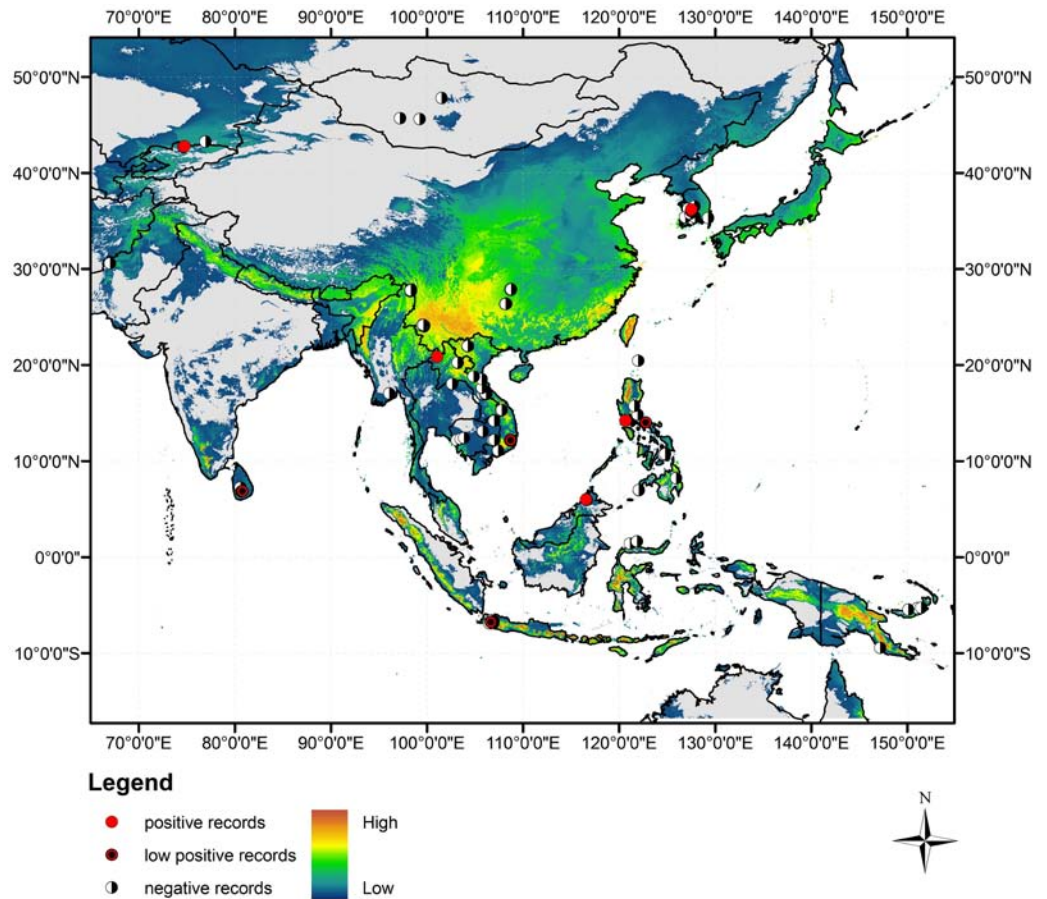


Figure 1. Map of predicted and observed *Batrachochytrium dendrobatidis* distribution in Asia. Map of Asia and Papua New Guinea showing Maxent predicted probability of (Bd) from low to high environmental suitability. Sample localities from field surveys are shown as black and white, red with black dot, and red circles indicating the highest level of Bd infection found.

fundamentally different in Asia relative to other regions. Our study, being the first major survey in Asia was not designed to conclusively answer that kind of question. We stress that though prevalence and disease intensity were low in our study, a Bd epidemic may still occur and that the potentially changing dynamics of Bd in Asia warrants additional research.

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